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The Effect of High-Pressure Treatment and Skimming on Caprine Milk Proteins

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Abstract: Background: Proteins are susceptible to HP-treatment and there is a need to determine the applicability of HP-treatment in dairy production. The aim of this study was to determine the effect of HP-treatment at 200–500 MPa ($t_{const.}$ = 10 min; $T_{const.}$ = 20 °C) and skimming of HP-treated milk on the content of nitrogen compounds and protein composition of caprine milk. Methods: The content of nitrogen (total, non-casein, non-protein) was determined using the Kjeldahl method. Casein fractions and whey proteins were separated using SDS-PAGE electrophoresis. Color parameters were measured in the CIELAB color space. Results: HP-treatment decreased ($p < 0.05$) the content of non-casein nitrogen and soluble whey proteins. Skimming decreased the content of nitrogen compounds, and the noted decrease was more pronounced in HP-treated milk. Pressure and skimming had no influence on the proportions of α-, β-, κ-casein, β-lactoglobulin and α-lactalbumin. Total color difference (ΔE) increased with a rise in pressure, particularly in skim milk. Conclusion: HP-treatment led to a loss of protein solubility at pH 4.6 in caprine milk. In HP-treated milk, skimming did not induce changes in protein composition, despite a decrease in the content of nitrogen compounds after the separation of the cream layer. Higher values of ΔE in skim milk than in whole milk point to changes in colloidal phase components.

Keywords: caprine milk; high-pressure treatment; nitrogen compounds; protein profile

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1. Introduction

Proteins are susceptible to the influence of high pressure and this determines the applicability of high-pressure treatment in dairy production [1–5]. High-pressure treatment weakens hydrophobic and electrostatic interactions, and it strengthens or weakens hydrogen bonds, depending on the applied pressure. Hydrogen bonds are damaged under exposure to ≥700 MPa. Chemical bonds can be arranged in the following descending order based on their sensitivity to high pressure: hydrophobic interactions > electrostatic interactions > hydrogen bonds > covalent bonds. High-pressure treatment affects mainly quaternary (hydrophobic and non-covalent interactions), tertiary (ionic and hydrophobic interactions) and secondary protein structures (intra- and intermolecular hydrogen bonds). Exposure to pressure higher than 200 MPa leads to the unfolding and reassociation of polypeptide chains. The pressure-resistant properties of proteins with a secondary structure (α-helices or β-sheets) are determined mainly by the presence of hydrogen bonds in molecular conformations [2–4,6–8].

High-pressure treatment induces the following changes in milk proteins: (1) the size of casein micelles changes [1,6,9–12]; (2) the proportions of soluble casein fractions

increase in the following order: κ~β > α_{s1} > α_{s2} [3,13,14]; (3) the soluble form of calcium and phosphorus content of milk increase [3,11,13,14]; (4) whey proteins are denatured in the following order: lactoferrin > β-lactoglobulin > immunoglobulins > serum albumin > α-lactalbumin [1,15]; and (5) milk proteins interact with milk fat globule membrane components [16].

Pressure-induced changes in protein structure influence the functional attributes of proteins, such as the ability to stabilize emulsions, bind water, and form gels. These changes are particularly important in the production of emulsions, ripened cheese, cottage cheese, and fermented milk products (such as yogurt), where the chemical, functional, and nutritional properties of proteins play a pivotal role [2–4].

Caprine milk casein is characterized by a higher content of β-casein and κ-casein, a lower content of α-casein, in particular α_{s1}-casein, in comparison with cow's milk [17–19]. Pressure-induced changes in milk proteins appear to be a crucial process in caprine milk. Caprine milk is more abundant in soluble casein than cow's milk [18], and β-casein in caprine milk is also dissociated under exposure to pressure or low temperature. The low hydration of casein micelles combined with the high content of ionized calcium and low citrate content contribute to the low colloidal stability of caprine milk [17,18]. High-pressure treatment induces the denaturation of caprine β-Lg, enhances protein retention in the curd [20,21], and increases the content of non-soluble minerals and proteins in caprine milk [13].

High-pressure treatment is applied in the food processing industry to prolong the shelf life of raw materials and food products. High-pressure technology offers an alternative to thermal preservation, pasteurization, and sterilization in dairy production, and it enables the development of novel products with desirable textures, flavors, and functional properties [2–5]. Most research studies investigating the effects of high-pressure treatment on proteins were conducted on cow's milk, whereas caprine milk proteins attracted less interest [3,11,13,20].

The aim of this study was to determine the effect of high-pressure treatment and skimming of HP-treated milk on the content of nitrogen compounds, protein composition, and color of caprine milk.

2. Materials and Methods

2.1. Research Materials

Raw milk for the experiment was obtained from Polish White Improved goats, aged 2–4 years, after winter kidding, in various stages of lactation. The animals were raised in a free-range system on permanent grassland and were housed at night. Additionally, goats received roughage (hay and clover) and concentrate (ground oats).

Raw milk was high-pressure treated (HP-treated) in the U4040 high-pressure chamber (Unipress Equipment, Warsaw, Poland) within a pressure range of 200–500 MPa (applied at 100 MPa intervals), at a constant time ($t_{const.}$ = 10 min) and constant temperature ($T_{const.}$ = 20 °C). Caprine milk was skimmed by centrifugation at 2000 rpm in the MPW-351 R centrifuge (MPW Med. Instruments, Warsaw, Poland) for 20 min at a temperature of 20 °C. Analytical samples were prepared according to the diagram in Figure 1. Untreated caprine milk was the control sample. The experiment was conducted in four independent replicates.

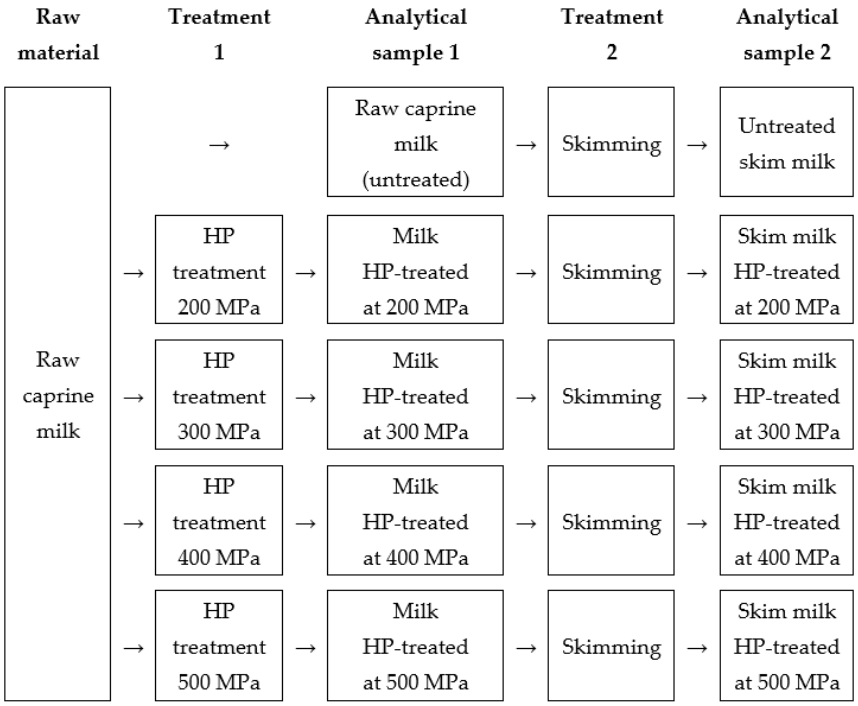

Figure 1. Preparation of analytical samples.

2.2. Nitrogen Compounds

Total nitrogen (TN) content and the content of non-casein nitrogen (NCN) and non-protein nitrogen (NPN) in whole and skim milk was determined by the Kjeldahl method [22]. The content of casein (CN = (TN − NCN) × 6.38) and whey protein (WP = (NCN − NPN) × 6.38) was determined. The results were expressed as the percentage content of nitrogen compounds (TN, NCN, NPN) and proteins (CN, WP) in whole milk and in skim milk relative to the content of nitrogen compounds and proteins in whole milk, respectively, before the separation of the cream layer.

2.3. SDS PAGE Electrophoresis

Proteins were separated in 30 µL of polyacrylamide gel (12% Resolving Gel, Mi ni-Protean TGX Precast Gels, Biorad, Life Science Group, Warsaw, Poland) in 10-well plates. Fresh samples (0.1 mL) were diluted with a sample buffer (0.9 mL) containing 10 mM Tris-HCl pH 6.8, 1.0% SDS, 20% glycerol, 0.02% bromophenol blue tracking dye, and 50 mM dithiothreitol, and were stored in a freezer. The samples were thawed at room temperature before analysis. The diluted samples in glass vials (1.5 mL, Bionovo, Eppendorf, Germany) were heated at 100 °C for 3 min in a steam chamber. Milk samples of 7 µL each were loaded onto SDS-PAGE gel.

Milk proteins were separated by electrophoresis in the Mini-PROTEAN® 3 Cell system (BIORAD, Hercules, PA, USA) with Thermo Scientific EC30000XL power supply (Shah Alam, Selangor, Malaysia). Electrophoresis was conducted at 150 V and 45 mA. Gels were run, stained and destained according to the procedure described by Verdi et al. [23]. Gels were scanned in the DNR LumiBis Bioimaging system (DNR Bio-Imaging Systems Ltd., Neve Yamin, Israel) to obtain the relative protein proportions in each sample. Scanned gels were analyzed in the Totallab program (BIO-RAD Laboratories, Inc., Hercules, CA, USA) to obtain relative protein proportions in each sample.

2.4. Color Analysis

Color parameters were measured in CIELAB color space with the CR-400 Chroma Meter (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA). Milk samples were placed in Petri dishes. The results were expressed as the total color difference between

the control sample and HP-treated samples and as the total color difference between untreated and HP-treated skim milk. The following equation was used in the calculations: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5}$, where L*—lightness from black (0) to white (100), a*—greenness (−) to redness (+), and b*—blueness (−) to yellowness (+).

2.5. Statistical Analysis

The effect of pressure (p), skimming (s), and the pressure and skimming (p × s) interaction on the analyzed parameters of caprine milk was determined by two-way ANOVA at a significance level of 0.05. Group means were compared in Fisher's LSD test. Data were processed in Statistica v. 13.0 software (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

The content of nitrogen compounds in caprine milk (Table 1) was similar to that reported in the literature [17,18,24]. Pressure (p) induced significant changes in total nitrogen content ($p < 0.05$), and the content of non-casein nitrogen ($p < 0.05$), casein ($p < 0.05$) and serum proteins ($p < 0.05$) in the analyzed milk samples, but it had no significant effect on the content of non-protein nitrogen. Skimming (s) exerted a significant decrease ($p < 0.05$) in the content of all nitrogen compounds. The pressure and skimming (p × s) interaction had a significant effect on total nitrogen content ($p < 0.05$), and the content of non-casein nitrogen ($p < 0.05$) (Table 1), casein ($p < 0.05$), and serum proteins ($p = 0.005$) (Table 2). Similar to pressure (p), the pressure and skimming (p × s) interaction had no significant influence on the content of non-protein nitrogen (Table 1).

Table 1. The effect of high-pressure treatment on the content of nitrogen compounds in whole and skim caprine milk (g/100 g of whole milk).

Milk		TN	NCN	NPN
Whole milk	0.1 MPa	0.515 ± 0.005 ^{a}	0.112 ± 0.001 ^{a}	0.034 ± 0.001 ^{a}
	200 MPa		0.096 ± 0.001 ^{c}	0.034 ± 0.001 ^{a}
	300 MPa		0.091 ± 0.003 ^{d}	0.034 ± 0.001 ^{a}
	400 MPa		0.089 ± 0.004 ^{d,e}	0.034 ± 0.002 ^{a}
	500 MPa		0.083 ± 0.003 ^{f}	0.034 ± 0.003 ^{a}
Skim milk	0.1 MPa	0.476 ± 0.007 ^{b}	0.102 ± 0.002 ^{b}	0.033 ± 0.003 ^{b}
	200 MPa	0.426 ± 0.023 ^{d}	0.087 ± 0.001 ^{e}	0.029 ± 0.001 ^{b}
	300 MPa	0.409 ± 0.001 ^{e}	0.084 ± 0.002 ^{f}	0.030 ± 0.001 ^{b}
	400 MPa	0.439 ± 0.011 ^{c,d}	0.081 ± 0.001 ^{f}	0.030 ± 0.001 ^{b}
	500 MPa	0.447 ± 0.007 ^{c}	0.078 ± 0.001 ^{g}	0.030 ± 0.001 ^{b}
Significance (p value)	p	0.000	0.000	NS
	s	0.000	0.000	0.000
	p × s	0.000	0.000	NS

Abbreviations: p, pressure; s, skimming; p × s, pressure and skimming interaction; TN, total nitrogen; NCN, non-casein nitrogen; NPN, non-protein nitrogen. Mean values ± SD; n = 4. Values with different superscripts in columns differ significantly at $p < 0.05$, depending on the pressure and skimming interaction; in column NPN, mean values with different superscripts differ significantly between whole and skim milk at $p < 0.05$; NS—not significant.

In whole milk, HP-treatment induced a significant decrease ($p < 0.05$) in the content of non-casein nitrogen, an increase ($p < 0.05$) in casein levels and a decrease ($p < 0.05$) in whey protein content, but it did not affect ($p \geq 0.05$) the content of non-protein nitrogen relative to the control sample. A significant decrease ($p < 0.05$) in the concentrations of non-casein nitrogen and whey proteins soluble at pH 4.6 was observed with a rise in pressure. In Fisher's LSD test, the average values of casein content in whole milk subjected to HP-treatment at 200–500 MPa formed a homogeneous group ($p \geq 0.05$) (Tables 1 and 2).

Table 2. The effect of high-pressure treatment on the content of casein and whey protein in whole and skim caprine milk (g/100 g of whole milk).

Milk		CN	WP
Whole milk	0.1 MPa	2.571 ± 0.038 ^{b}	0.502 ± 0.009 ^{a}
	200 MPa	2.675 ± 0.034 ^{a}	0.397 ± 0.008 ^{c}
	300 MPa	2.706 ± 0.019 ^{a}	0.366 ± 0.015 ^{d}
	400 MPa	2.720 ± 0.014 ^{a}	0.352 ± 0.017 ^{d,e}
	500 MPa	2.758 ± 0.019 ^{a}	0.314 ± 0.017 ^{f,b}
Skim milk	0.1 MPa	2.388 ± 0.048 ^{c}	0.442 ± 0.015 ^{b}
	200 MPa	2.162 ± 0.137 ^{e}	0.369 ± 0.008 ^{d}
	300 MPa	2.074 ± 0.015 ^{f}	0.344 ± 0.012 ^{e}
	400 MPa	2.286 ± 0.077 ^{d}	0.323 ± 0.008 ^{f}
	500 MPa	2.356 ± 0.052 ^{c,d}	0.304 ± 0.008 ^{g}
Significance (p value)	P	0.000	0.000
	S	0.000	0.000
	p × s	0.000	0.005

Abbreviations: p, pressure; s, skimming; p × s, pressure and skimming interaction; CN, casein; WP, whey protein. Mean values ± SD; n = 4. Values with different superscripts in columns differ significantly at $p < 0.05$, depending on the pressure and skimming interaction.

The present study revealed that HP-treatment affects the content of nitrogen compounds in caprine milk (Table 2). The observed decrease in whey protein levels suggests that whey proteins are denatured and enter into interactions with casein. HP-induced denaturation of whey proteins leads to loss of their solubility at pH 4.6 after previous interactions with casein in caprine milk [11,20] and in bovine milk [6]. HP-treatment denatures up to 80% of β-lactoglobulin in bovine milk [13,25,26] and caprine milk [11,20]. According Nassar et al. [27], the degree of whey protein denaturation in caprine milk treated at 500 MPa reached 54.1% after one day of storage at temperature of 4 °C.

In skim milk obtained from the control sample and from HP-treated samples subjected to different pressure, nitrogen compounds were transferred from whole milk to the cream layer after centrifugation. The above was confirmed by Fisher's LSD test which revealed that the average content of the analyzed nitrogen compounds tended to decrease ($p < 0.05$) in untreated and HP-treated skim milk relative to the corresponding samples of whole milk. The concentration of nitrogen compounds was higher ($p < 0.05$) in untreated skim milk than in HP-treated skim milk. Subject to the applied process parameters, HP-treatment decreased ($p < 0.05$) the content of whey proteins in skim milk with a rise in pressure and decreased total nitrogen content and the content of casein in skim milk which reached minimum values at 300 MPa ($p < 0.05$) (Tables 1 and 2). Liquid-phase whey proteins consist mainly of β-lactoglobulin, α-lactalbumin and, to a smaller extent, serum albumin, immunoglobulins, lactoferrin, transferrin, ferritin, proteose peptone, calmodulin, prolactin and folate-binding protein. Non-protein nitrogen compounds include ammonia, urea, creatinine, creatine, uric acid and amino acids [18].

The decrease in the protein content of milk after centrifugation can probably be attributed to the interactions between milk proteins and emulsion phase components. In a study by Ye et al. [16], β-lactoglobulin, α-lactalbumin and κ-casein were associated with milk fat globule membranes in cow's milk under exposure to >100 MPa, ≥700 MPa and ≥500 MPa, respectively.

The effect of HP-treatment on whole milk proteins and protein distribution after the separation of the cream layer was determined by electrophoresis. The protein profile of caprine milk samples is presented in Figure 2. Casein fractions, including α-casein (α-CN), β-casein (β-CN) and κ-casein (κ-CN), and whey proteins, including α-lactalbumin (α-LA) and β-lactoglobulin (β-LG), were identified and quantified in whole milk and skim milk. The results of electrophoretic separation were used to identify proteins with a molecular weight of ≥40 kDa, including milk fat globule membrane proteins [28].

Electrophoretic bands denoting proteins with a molecular weight of ≥40 kDa were less intense in skim milk after cream separation (Figure 2, bands 6–10). Proteins with a molecular weight of ≥40 kDa were not quantified. The presence of proteins with a molecular weight higher than that of β-lactoglobulin and lower than that of κ-casein was observed in HP-treated milk.

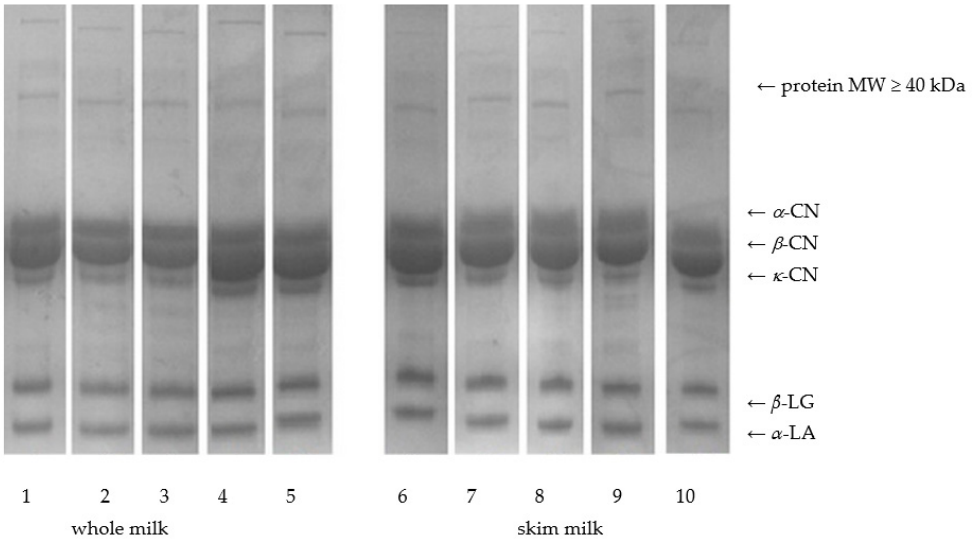

Figure 2. Electrophoresis on polyacrylamide gel (SDS-PAGE) under reducing conditions. Proteins in whole caprine milk: untreated milk (1); HP-treated milk at p = 200 MPa (2), p = 300 MPa (3), p = 400 MPa (4), p = 500 MPa (5). Proteins in skim caprine milk: untreated milk (6); HP-treated milk at p = 200 MPa (7), p = 300 MPa (8), p = 400 MPa (9), p = 500 MPa (10).

The results of densitometric analyses of the protein profile are presented in Table 3. The densitometric quantification of electrophoretic bands in untreated caprine milk revealed the highest content of β-CN, whereas the concentrations of the remaining proteins were arranged in the following descending order: α-CN > β-LG > κ-CN~α-LA (Figure 2 band 1; Table 3). The composition of caprine milk proteins is determined by breed, lactation stage and diet [29,30]. In a study by Abbas et al. [29], the proportions of casein fractions α_{s1}-CN, α_{s2}-CN, β-CN and κ-CN in caprine milk were determined at 1:2:5:2. Casein fractions α_{s1}, α_{s2}, β+γ-CN and κ-CN accounted for 10.4, 14.5, 62 and 13.2% of total casein in caprine milk, respectively. According to Hejtmankowa et al. [30], caprine milk contains 2.75% protein, 0.433% whey protein, and 0.119% of β-LG, with an average β-LG/α-LA ratio of 0.59.

A quantitative analysis of proteins in HP-treated whole milk also revealed the highest concentration of β-CN, smaller amounts of α-CN, and the lowest content of κ-CN (Table 3). The results of two-way ANOVA indicate that pressure (p), skimming (s), and the pressure and skimming (p × s) interaction did not influence the content of α-CN, β-CN, κ-CN, α-LA and β-LG ($p \geq 0.05$). Two-way ANOVA revealed that the casein to whey protein ratio was significantly ($p < 0.05$) influenced by pressure (p). Skimming (s) and the pressure and skimming interaction (p × s) had no effect on the casein to whey protein ratio. The casein to whey protein ratio was higher in milk treated at 500 MPa than in untreated milk and milk subjected to 200–400 MPa. A similar relationship was observed in skim milk obtained from whole untreated milk and in skim milk obtained from pressurized whole milk. Skimming (s) and the pressure and skimming interaction (p × s) did not exert a significant influence on the casein to whey protein ratio (Table 4).

Table 3. The effect of high-pressure treatment on the content of α-casein (α-CN), β-casein (β-CN), κ-casein (κ-CN), β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) in whole and skim caprine milk (g/100 g of protein).

Milk		α-CN	β-CN	κ-CN	β-LG	α-LA
Whole milk	0.1 MPa	24.85 ± 0.59	34.68 ± 1.44	13.07 ± 0.99	14.40 ± 1.49	13.00 ± 0.27
	200 MPa	24.81 ± 0.65	34.41 ± 1.19	12.47 ± 1.28	14.81 ± 1.05	13.49 ± 0.50
	300 MPa	24.85 ± 0.63	34.23 ± 2.31	12.72 ± 0.60	14.60 ± 0.56	13.60 ± 0.82
	400 MPa	24.79 ± 0.92	34.38 ± 2.33	12.82 ± 1.23	14.39 ± 0.64	13.62 ± 0.55
	500 MPa	24.74 ± 1.41	36.21 ± 3.27	12.61 ± 1.04	13.67 ± 1.48	12.77 ± 1.15
Skim milk	0.1 MPa	24.70 ± 2.22	32.66 ± 1.73	12.86 ± 0.58	15.96 ± 0.46	13.82 ± 0.67
	200 MPa	23.83 ± 0.22	34.02 ± 0.80	12.81 ± 0.66	15.47 ± 1.27	13.88 ± 0.59
	300 MPa	24.22 ± 0.46	34.88 ± 0.80	12.40 ± 0.96	15.04 ± 0.79	13.45 ± 0.89
	400 MPa	24.68 ± 1.07	34.25 ± 0.76	12.41 ± 0.82	14.80 ± 0.44	13.86 ± 0.77
	500 MPa	26.35 ± 0.84	35.84 ± 0.88	11.48 ± 0.83	13.41 ± 0.98	12.92 ± 0.85
Significance (p value)	P	NS	NS	NS	NS	NS
	S	NS	NS	NS	NS	NS
	p × s	NS	NS	NS	NS	NS

Abbreviations: p, pressure; s, skimming; p × s, pressure and skimming interaction; NS—not significant. Mean values ± SD; n = 4.

Table 4. The effect of high-pressure treatment on the content of casein (CN), whey protein (WP) and the CN:WP ratio in whole and skim caprine milk (g/100 g of protein).

Milk		CN (α- + β- + κ-CN)	WP (β-LG + α-LA)	CN:WP
Whole milk	0.1 MPa	72.60 ± 1.67 ^{a}	27.40 ± 1.67 ^{a}	2.66 ± 0.22 ^{b}
	200 MPa	71.70 ± 0.55 ^{a}	28.30 ± 0.55 ^{a}	2.53 ± 0.07 ^{b}
	300 MPa	71.80 ± 1.08 ^{a}	28.20 ± 1.08 ^{a}	2.55 ± 0.14 ^{b}
	400 MPa	71.99 ± 0.37 ^{a}	28.01 ± 0.37 ^{a}	2.57 ± 0.05 ^{b}
	500 MPa	73.56 ± 2.45 ^{b}	26.44 ± 2.45 ^{b}	2.80 ± 0.36 ^{a}
Skim milk	0.1 MPa	70.22 ± 1.02 ^{a}	29.78 ± 1.02 ^{a}	2.36 ± 0.12 ^{b}
	200 MPa	70.65 ± 1.06 ^{a}	29.35 ± 1.06 ^{a}	2.41 ± 0.12 ^{b}
	300 MPa	71.50 ± 0.81 ^{a}	28.50 ± 0.81 ^{a}	2.51 ± 0.10 ^{b}
	400 MPa	71.34 ± 1.07 ^{a}	28.66 ± 1.07 ^{a}	2.49 ± 0.13 ^{b}
	500 MPa	73.67 ± 0.82 ^{b}	26.33 ± 0.82 ^{b}	2.80 ± 0.12 ^{a}
Significance (p value)	P	0.018	0.018	0.017
	S	NS	NS	NS
	p × s	NS	NS	NS

Abbreviations: p, pressure; s, skimming; p × s, pressure and skimming interaction. Mean values ± SD; n = 4. Values with different superscripts in the CN column, WP column and the CN:WP column differ significantly at $p < 0.05$, depending on the applied pressure; NS—not significant.

The results of electrophoresis performed under reducing conditions revealed that HP-treatment at ≤400 MPa did not affect the content of casein fractions (α, β and κ), α-lactalbumin, β-lactoglobulin (Table 3) or the casein to whey protein ratio (Table 4). The protein content of HP-treated milk decreased after skimming (Table 2), but the proportions of the analyzed milk proteins remained unchanged (Table 3). In the present study, the protein profile determined by electrophoretic separation did not support the identification of proteins that interacted with the milk fat globule membrane.

Pressure (p), skimming (s) and the pressure and skimming (p × s) interaction significantly ($p < 0.05$) influenced the total color difference ΔE between whole and skim milk. The values of ΔE increased with a rise in pressure (Figure 3). The pressure-induced changes in color of whole milk, increasing with a rise in pressure, were reported by Gervilla et al. [31] and Kiełczewska et. al. [32].

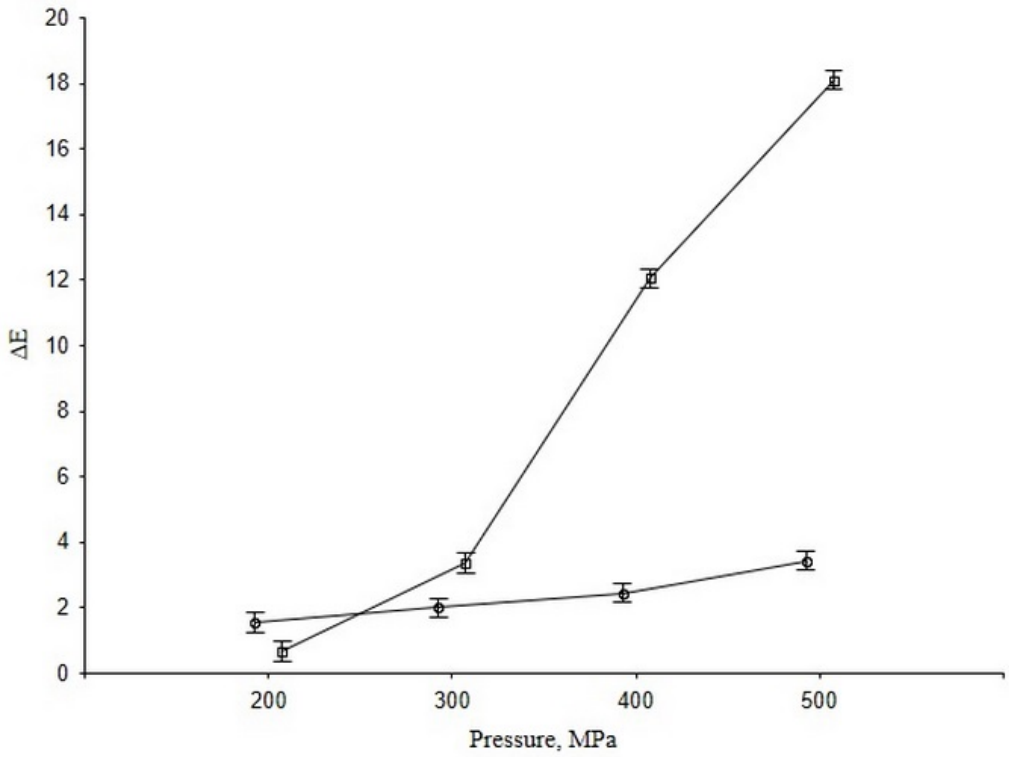

Figure 3. The effect of high-pressure treatment on the total color difference ΔE in whole milk (open circle) and skim milk (open square). Vertical bars denote 0.95 confidence intervals. Significance: pressure effect at $p < 0.05$; skimming effect at $p < 0.05$; interaction effect of pressure and skimming at $p < 0.05$.

The values of ΔE were lower in skim milk HP-treated at 200 MPa than in the corresponding sample of whole milk. The highest values of ΔE were noted in skim milk obtained from milk HP-treated at ≥300 MPa (Figure 3). ΔE values were higher in skim milk than in whole milk HP-treated at ≥300 MPa, which indicates that differences in color resulted mainly from changes in colloidal phase components.

Changes in the color of milk can be attributed to the modification of colloidal and emulsion phase components, namely changes in the size of casein micelles that undergo disaggregation and aggregation [33], as well as changes in the number and size of milk fat globules and their aggregates [34]. The increase in the total color difference of HP-treated skim milk was indicative of changes in the colloidal milk phase, in particular under exposure to ≥300 MPa. The main caprine milk proteins are casein and its fractions: αs1-casein αs2-casein, β-casein and κ-casein, which, together with colloidal calcium phosphates, are the structural components of casein micelles. Literature data partly explain the influence of HP-treatment at ≥300 MPa on the total color difference in skim caprine milk. According to research, the decrease in the lightness of HP-treated milk resulted mainly from changes in the size of protein molecules and casein disaggregation into submicelles at <150 MPa [4,5]. HP-treatment (>100 MPa) increased casein solubility [9,14,25,26] and induced changes in the size of casein micelles which are influenced by process parameters [5,12]. The disintegration of casein micelles and an increase in the solubility of different fractions (e.g., *β*-casein) is determined by the number of phosphoseryl residues, and it is influenced by the dissociation of colloidal calcium phosphate and the weakening of hydrophobic interactions. Subject to the applied pressure, HP-treatment decreases the size of casein micelles (200 MPa), induces differences in the size of casein micelles due to their fragmentation and the re-aggregation of subunits, promotes hydrophobic interactions that lead to the formation of micelles larger than the native micelles (250 MPa), and decreases the size of casein micelles (≥300 MPa), in particular under exposure to lower temperature [9,12,14].

Pressure-induced changes in the composition and processing suitability of caprine milk proteins exert a significant influence on the properties of dairy products. Changes in protein structure, including pressure-induced denaturation of whey proteins, and the formation of whey protein and casein aggregates, influence the functional attributes of proteins, such as the ability to stabilize emulsions, bind water and form gels. Proteins determine the properties of both raw milk and dairy products; therefore, pressure-induced changes in protein structure are an important consideration in industrial processes. These changes are particularly important in the production of emulsions, ripened cheese, cottage cheese and fermented milk products (such as yogurt), where the chemical, functional and nutritional properties of proteins play a pivotal role [1–5].

4. Conclusions

High-pressure treatment exerted a varied influence on the content of nitrogen compounds, excluding non-protein nitrogen. HP-treatment decreased the content of whey proteins soluble at pH 4.6, which could be attributed to their denaturation and interactions with casein. HP-treatment promotes the formation of proteins with a molecular weight higher than that of β-lactoglobulin and lower than that of casein. Despite the above, HP-treatment did not affect the percentage content of α-CN, β-CN, κ-CN, β-LG or α-LA in total protein. The observed changes in the dynamic equilibrium of HP-treated proteins can affect the processing suitability of caprine milk. Skimming decreased the content of nitrogen compounds in the control sample, and the resulting decrease was more pronounced in HP-treated samples. In HP-treated milk, skimming did not induce changes in the proportions of casein fractions or whey protein (relative to the corresponding samples of whole milk), despite a decrease in the content of all nitrogen compounds after the separation of the cream layer. HP treatment induced changes in colour in milk. Total color difference in milk increased with pressure, especially in skim milk. Skimming induced changes in milk color relative to the corresponding samples of whole milk. Changes in the values of total color difference in milk were result of the modification of the colloidal phases, mainly change in size of casein micelles. Further research is needed to determine the nature of interactions between proteins and modification of proteins' structure in HP-treated caprine milk. The presented results may be useful in designing new processing lines, which is an important consideration in view of the growing interest in the diversification of raw materials in the dairy industry.

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